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Key Message: "Increasing knowledge about the molecular characteristics of mesothelioma had led to the identification of novel potential targets for systemic therapy. This review elaborates on the rationale behind targeted approaches that have been and are undergoing exploration in mesothelioma and summarizes available clinical results and ongoing efforts to improve the systemic therapy of mesothelioma."

Abstract

Malignant mesothelioma is an incurable disease associated with asbestos exposure arising in the pleural cavity and less frequently in the peritoneal cavity. Platinum-based combination chemotherapy with pemetrexed is the established standard of care. Multimodality approaches including surgery and radiotherapy are being investigated. Increasing knowledge about the molecular characteristics of mesothelioma had led to the identification of novel potential targets for systemic therapy. Current evidence suggests pathways activated in response to merlin deficiency, including Pi3K/mTOR and the focal adhesion kinase, as well as immunotherapeutic approaches to be most promising. This review elaborates on the rationale behind targeted approaches that have been and are undergoing exploration in mesothelioma and summarizes available clinical results and ongoing efforts to improve the systemic therapy of mesothelioma.

Key words: mesothelioma, targeted therapy, immunotherapy,

Introduction

Mesothelioma is a fatal disease predominantly arising in the pleural cavity and less so in the peritoneal cavity. The association of mesothelioma with asbestos exposure is well established. The time from exposure to the diagnosis is on the average greater than 40 years, explaining why the disease incidence is still raising in many countries despite working bans on the use of asbestos in the early 1990s. Platinum-based chemotherapy, mostly combined with the folate antagonist pemetrexed, is the established standard of care [1]. In earlier stages of pleural mesothelioma multimodality therapy including extrapleural pneumonectomy or more recently extended pleurectomy/decortication, with or without radiotherapy, are being investigated in selected patients [2]. There is currently no defined standard for second line therapy. The rationale behind investigating novel targeted approaches, available results and ongoing efforts are summarized in this review.

Exploring molecular alterations

Data mining of version 71 of the catalogue of somatic mutations in cancer (COSMIC, http://www.sanger.ac.uk/cosmic) reveals that the genes that are most frequently mutated in malignant pleural mesothelioma are *cyclin-dependent kinase inhibitor 2A* (*CDKN2A), neurofibromatosis type 2* (*NF2)* and *BRCA-associated protein 1.*

Targeting the cell cycle:
Mesothelioma lack expression of both *CDKN2A* encoded proteins p16 and ARF due to gene deletion or methylation (reviewed in [3]). Deletion in *CDKN2A* leads to loss of control of cyclin D-dependent kinases (CDK). Although CDK4/6 specific inhibitors are under investigation in clinical trials, animal models with CDKN2A deficiency showed that loss of CDKN2A function is not necessarily associated with CDK4/6 addiction [4].

Although only a minor fraction of mesothelioma presents with p53 mutations [5], this lead to the hypothesis that this tumor might be dependent on G2 checkpoint and therefore vulnerable to a G2 checkpoint inhibition when combined with chemotherapy. In line with this hypothesis, the calmodulin-binding peptide (CBP501) was clinically tested in combination with cisplatin and pemetrexed in order to increase the sensitivity of mesothelioma cells to chemotherapy [6]. In patients receiving CBP501 with chemotherapy PFS of more than 4 months was achieved compared to 39% receiving chemotherapy alone (Table 1) [7]

Targeting NF2/Hippo deficiency:

The NF2/Hippo signalling pathway has been shown to be disrupted in most mesothelioma [5][8] characterized by mutation or inactivation of the *NF2* gene (reviewed in [3]). Experimental animal models indicate that this event, together with a deficiency in cyclin-dependent kinase activator inhibitor (*CDKN2A)*, is essential for mesothelioma development. Therefore, targeting molecules involved in the NF2/Hippo pathway is of major interest (Figure 1) for the treatment of mesothelioma. NF2 is an upstream regulator of the so-called Hippo signalling cascade, [9] which controls the transcriptional co-activator Yes-associated protein (YAP)). The dysfunction of Hippo pathway, which leads to increased YAP activity [10], induces oncogenic transformation by the activation of transcription factors including transcription enhancers activation domain (TEAD) family members [11]. Upon binding TEADs, YAP/TAZ up-regulates the expression of several growth promoting factors. YAP is constitutively active in more than 70% of primary mesotheliomas [8], [12]. Hedgehog signalling has a role in maintaining YAP protein stability in progenitor cells [13] and is activated in mesothelioma, consistent with the re-activation of a signalling known to be essential during embryonic mesothelial development [14]. Treatment of mesothelioma xenografts with the hedgehog antagonist HhAntag led to a decrease of the tumour volume accompanied by a decrease in Ki-67 labelling index.

Another possible approach is the direct inhibition of YAP activity. Three compounds related to porphyrin that could inhibit the transcriptional activity of YAP in vitro were

identified by screening of a Johns Hopkins Drug Library [15]. One of these, verteporfin, in clinical use as a photosensitizer in photocoagulation therapy for macular degeneration, was moderately effective at blocking mouse *Yap1*-overexpression- or loss of *Nf2*-driven hepatic tumorigenesis. These data suggest further investigation of these compounds as anticancer therapies.
NF2 suppresses tumorigenesis by migrating into the nucleus where it inhibits the E3 ubiquitin ligase CRL4 and through that controls a subset of Hippo pathway target genes [16], therefore CRL inhibitors such as MLN4924 should be investigated in mesothelioma.

Interestingly, expression of constitutively active YAP causes widespread miRNA suppression [17]. Thus, the Hippo pathway may be responsible for the widespread miRNA repression observed in cancer, including mesothelioma. To overcome the difficulties of directly delivering miRNA mimics, minicells composed of achromosomal bacterial cells and targeted by bispecific antibodies, have been developed. These have been used to restore miR16 and induce growth arrest in mesothelioma xenografts [18]. Minicells can be given safely to patients with advanced cancer [19] and a clinical trial has started in mesothelioma (ACTRN12614001248651).

Targeting NF2 associated cytoskeletal alterations:
In a systematical screen for tumor suppressors whose functional inactivation would result in microtubular instability, NF2 was identified as a microtubule stabilizer [20], demonstrating that the microtubular network might be significantly affected in mesothelioma. Based on this and in vitro studies, epothilone B would be a candidate for clinical evaluation [21] .

NF2 alterations result also in activation of the focal adhesion kinase (FAK) and merlin deficiency predicts for sensitivity to FAK inhibitors [22, 23]. The underlying mechanism is that survival and proliferation signals seem mediated through extracellular matrix-integrin signals promoting FAK activation in mesothelioma cells with inactivating NF2 mutations [23]. There is a phase I and two phase II trials ongoing testing the FAK

inhibitors GSK2256098 and defactinib (VS-6063) in mesothelioma (Table 1). Determination of the NF2 status in these trials will allow exploring whether the clinical outcome is indeed associated with alteration of NF2.

Targeting PI3K/mTOR

PI3K/mTOR signalling is activated in mesothelioma [24]. For the time being the reason for this has not been elucidated, as neither PI3K nor receptor tyrosine kinase mutations/amplifications have been found in two recent high-throughput studies [5] [25]. NF2-null cells were shown sensitive to growth inhibitory effects of rapamycin [26] via mechanisms involving PI3K signalling-independent mammalian target of rapamycin complex (mTORC1) activation. However, preliminary results of a phase II trial [27] of the mTOR inhibitor, everolimus, did not show to be active in unselected MPM patients (Table 1). In addition, a peritoneal mesothelioma model was recently generated by deficiency in p53 and the tuberous sclerosis gene, a negative regulator of mTORC [28]. GDC-0980 is a small molecule inhibitor of class I PI3K and mTOR (mTORC1 and mTORC2) [29] has been tested in phase I studies (Table 1) and the preliminary result of the phase I extension cohort showed two objective responses among 26 patients with mesothelioma [30]. Another dual PI3K/mTOR inhibitor, LY3023414 is tested in a phase I trial (Table 1).

Synthetic lethal approaches

A large proportion of mesothelioma [31] show reduced expression of arginosuccinate synthetase-1, the rate-limiting enzyme for arginine biosynthesis, rendering cells auxotrophic for arginine and consequently susceptible to the arginine degrading enzyme arginine deiminase (Adi-PEG20). Preliminary results of a randomized phase II study (Table 1) showed a significant PFS improvement delivering ADI-PEG20 versus best supportive care [32]. Almost all epithelioid and about 50% of sarcomatoid mesothelioma express calretinin [33] which is widely used as a robust diagnostic mesothelioma marker. Since its silencing inhibits mesothelioma cell survival in vitro, this may offer another opportunity for a therapeutic intervention [34] .

Tyrosine Kinase Inhibitors (TKIs):

Deregulated expression of growth factors or proteins involved in downstream signalling pathways has been shown to play an important role in malignant transformation of mesothelial cells. Molecular studies in malignant pleural mesothelioma have confirmed that growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor receptor beta (PDGFRβ) and the epidermal growth factor receptor family are frequently activated. Several clinical trials have tried to exploit these specific characteristics using tyrosine kinase inhibitors.

Multitarged TKIs

Overexpression of platelet-derived growth factor (PDGF) has been observed and found to be associated with a poor prognosis [35]. While normal mesothelial cells express predominantly the PDGFRα subunit and less PDGFRβ, mesothelioma was shown to overexpress PDGFRβ [36]. In vitro studies VEGF stimulates the growth of mesothelioma cells and anti-VEGF rabbit polyclonal antibodies inhibit their growth [37-39].

The co-expression of c-kit in 26% [40] of mesothelioma has inspired the use of imatinib, an inhibitor of bcr/abl, c-kit and PDGFRα and β. Four phase II clinical trials of imatinib as a single agent in mesothelioma have been published. Of a total of 94 patients treated, no objective response was seen and progression free survival was less than two months [41, 42], [43, 44] .

Sorafenib is a potent inhibitor of VEGFR2, VEGFR3, Raf, PDGFRβ and c-kit. In a phase II trial with 50 patients evaluable for response three partial responses and 27 disease stabilizations were observed, results deemed insufficient for further evaluation of sorafenib [45]. Another phase II trial assessed sorafenib in a single-arm phase II study enrolling 53 patients using a Simon's two-stage design. Treatment was well tolerated and demonstrated a moderate activity with a median PFS of 5·1 months [46] .

Sunitinib, a VEGFR1, VEGFR2, VEGFR3, PDGFRβ, and c-kit TKI was tested in a phase II trial in 53 patients resulting in partial responses in 6 and stable disease in 34. An accompanying biomarker study was unsuccessful [47]. Another phase II trial using a Simon's two-stage design and a primary outcome of objective response rate did not meet the criteria for continuing to the second stage of accrual, with only one partial response observed among 35 patients [48] . A phase I trial with an expansion cohort in mesothelioma patients demonstrated that sunitinib was not well tolerated at 37·5 mg with standard pemetrexed and cisplatin doses, requiring dose reductions mainly due to cumulative myelosuppression and subsequent limited activity [49] .

Pazopanib, a broad anti-angiogenic broad TKI targeting VEGFR1-3, PDGFRα and β, and c-kit, has been evaluated in a phase II trial as a single agent single agent in 34 mesothelioma patients resulting in a six months progression-free survival of 48% (Table 1). Vatalanib targets VEGFR1, VEGFR2, c-kit, PDGFRβ and c-Fms. It was tested in a phase II trial and did not achieve the protocol-specified three-months PFS, ending its development mesothelioma [50]. Cediranib is a VEGFR 1-3 TKI. Two phase II trials were able to show only a modest single-agent activity with partial remission in four and five patients out of in 54 and 51 patients respectively, however with significant toxicities [51, 52] .

Dasatinib is an inhibitor of the Src family of non-receptor tyrosine kinases and PDGFRβ. Single-agent dasatinib did not show any activity in mesothelioma and was associated with inacceptable pulmonary toxicities in a phase II trial enrolling 46 patients [53] .

While the results from most of these trials were considered as negative, the fact remains that activity of cediranib, imatinib, sunitinib or sorafenib was observed in a low proportion of patients, suggesting a need for the identification of predictive biomarkers to support further development. However, given the multitargeted nature of these TKIs and the difficulties encountered in identifying biomarkers for anti-angiogenic therapies in general this will unlikely be successful.

Due to the few responses to TKIs, combinatorial regimens with chemotherapy are ongoing. To this end, a study with cediranib in combination with chemotherapy is currently recruiting (Table 1) and another trial phase I/II trial has been randomizing patients to cisplatin and pemetrexed with or without axitinib, a pan-VEGFR inhibitor (Table 1). Several trials combining chemotherapy with multitargeted TKIs are still being discussed or currently in phase I (Table 1).

Restricted TKI

Erlotinib and gefitinib are first generation TKIs targeting specifically EGFR. EGFR expression has been demonstrated by immunohistochemistry in 70% - 95% of mesothelioma specimens and its overexpression might be associated with a favourable prognosis [54-56]. Despite some encouraging in vitro data, phase II trials in patients with untreated mesothelioma using gefitinib or erlotinib have failed to demonstrate significant activity. Gefitinib demonstrated partial remissions in two out of 43 untreated patients [56]. Erlotinib was ineffective in 63 untreated patients despite high expression of EGFR in patients' tumors. Here, the activation of the PI3K/Akt downstream pathways was proposed as a potential mechanism of primary resistance [57]. Also the combination of erlotinib and bevacizumab after platinum-based chemotherapy did not result in any responses among 24 mesothelioma patients [58] .
Other targets including MET- and FGFR3-TKIs, tiventanib and dovitinib, are under investigation (Table 1).

Histone deacetylase inhibitors

The equilibrium between the acetylated or deacetylated forms of histone proteins is regulated by histone acetyltransferase and histone deacetylase (HDAC). HDAC inhibitors will alter the wrapping DNA around histones, modify the access of transcription factors and consequently impact the expression of various genes.

After two promising phase I trials including small numbers of mesothelioma treated by vorinostat as monotherapy or combined with chemotherapy, [59] a placebo-controlled

phase III trial including 660 mesothelioma patients who had progressed after treatment with pemetrexed and platinum was launched. Results were reported at ECCO-ESMO 2011 and were negative for all endpoints [60] . Another small phase II trial with the HDAC inhibitor belinostat was also negative [61].

In vitro data suggested valproic acid might have a proapoptotic effect in synergy with doxorubicine. A phase II trial evaluating valproic acid in combination with doxorubicine in 45 patients pre-treated with chemotherapy demonstrated objective responses in seven with a median progression-free survival of 2·5 months [62].

Proteasome Inhibitors

Bortezomib was found to be inactive in a single-arm phase II trial in poor performance-status first-line and second-line mesothelioma patients with only one confirmed response of 33 patients [63]. Bortezomib was also evaluated in combination with cisplatin in a prospective phase II trial with progression-free survival rate at 18 weeks as primary end-point [64]. 82 patients were treated with an 18 weeks progression-free survival of 53%. Based on statistical assumptions, the null hypothesis could not be rejected and the combination was considered not worthy of further investigation.

Bevacizumab

A randomized phase II trial of untreated mesothelioma patients compared cisplatin-gemcitabine alone or with bevacizumab. The addition of bevacizumab did not improve response, progression-free survival, or overall survival compared to chemotherapy alone. A potential benefit in patients with low circulating levels of VEGF was suggested in subgroup analysis [65]. Another phase II trial combined treatment of cisplatin and pemetrexed with bevacizumab in 45 inoperable chemotherapy naïve mesothelioma patients. Response rate of 41%, median PFS of 6·9 months, and median OS 15·3 months were reported, with development of hypertension as a possible surrogate

marker for bevacizumab activity [66].

A two-armed phase II/III trial compared the standard of care cisplatin and pemetrexed regimen with or without bevacizumab as first-line treatment and maintenance in inoperable mesothelioma patients. While tolerance was good, the preliminary analysis of the study revealed that disease control at six months favoured the bevacizumab arm (73·5% and 43·2%, P = 0·010). Final results of this trial are eagerly awaited [67].

Other anti-angiogenic interventions

Vascular Disrupting Agents

BNC105P is a small molecule inhibiting tubulin polymerisation that functions as a vascular disrupting agent through selectively shutting down tumor blood vessels without affecting normal vasculature. Preclinical models have demonstrated significant tumor growth suppression and regression with BNC105P [68] .VDA BNC105P was tested as a second line treatment in advanced mesothelioma and proven ineffective in a trial of 30 patients [69].

Thalidomide

Thalidomide is the oldest and perhaps the most extensively studied drug classified as an anti-angiogenic agent, which activity is attributed to the inhibition of VEGF, basic fibroblast growth factor, as well as TGFβ and TNFα [70, 71]. A phase I trial explored its activity in 40 mesothelioma patients, a third of them being treatment naive. There were no responders, with an OS of 7·6 months and eleven were free of progression after six months [72]. Two parallel unpublished phase II studies evaluated thalidomide in combination with gemcitabine/cisplatin or thalidomide as a single agent. Thirty-one chemotherapy naïve patients received thalidomide and gemcitabine/cisplatin with partial responses in 14% and an OS of 11 months [73]. Twenty-seven patients pre-treated or unsuitable for chemotherapy were treated with single agent thalidomide. Responses occurred in 6% of the patients, and OS was 11 months.

The utility of thalidomide in mesothelioma as maintenance therapy for up to one year was evaluated in a large randomized phase III trial in 222 patients who had not progressed after four to six cycles of pemetrexed with or without platinum. The primary endpoint of of time to progression was of 3.6 months in the experimental arm as compared to 3·5 months in the placebo arm, demonstrating the absence of benefit of thalidomide maintenance [74].

NGR-hTNF

NGR-hTNF consists of human tumor necrosis factor alpha (hTNF-alpha) fused to the tumor-homing peptide asparagine-glycine-arginine (NGR) able to selectively bind an aminopeptidase N isoform overexpressed on tumor blood vessels. Based on an exploratory phase II trial in mesothelioma a phase III trial comparing NGR-hTNF to best supportive care is ongoing [75] .

Immunotherapeutic approaches

In mesothelioma, a chronic inflammatory response represented by infiltrating lymphocytes and plasma cells was associated with a better prognosis [76]. As in other tumors, immunotherapeutic strategies aimed at balancing the immune system in favour of an anti-mesothelioma response are being explored (Figure 2).

Transforming growth factor β

Transforming growth factor β (TGF-β) is a pleiotropic cytokine which can be produced by cancer, stroma and immune cells [77-79] .TGF-β attenuates the anti-tumor immune response blocking priming and effector phase of tumor-specific T cells. Fresolimumab (GC1008), a humanized monoclonal antibody neutralizing active forms of TGF-β, has been examined in a phase I trial in 13 patients with mesothelioma [80]. No objective response was seen, but three patients had stable disease at three months and five patients developed an enhanced antibody response to mesothelioma lysates (Table 2).

Interferon-β/interferon–α

Interferon-β (IFN-β) is type 1 cytokine with multiple functions resulting in antiviral, anti-proliferative, anti-angiogenic activity and immune cell stimulation. IFN-β has been delivered by an adenoviral vector into the pleural effusion of patient with mesothelioma in two phase I trials, using one or two injections [81, 82]. Both trials found a transient increase of IFN-β in the pleural cavity, mitigated by a neutralizing antibody response resulting in clearance of the adenovirus and decrease of IFN-β. There were no safety issues and antibody responses against the mesothelin could be induced in some patients. Of the 13 patients treated four had stable disease as best response.
IFN-α can promote the differentiation and activity of host immune cells and moreover correlates with generation of a durable antitumor response [83]; one clinical study exploring the efficacy of this cytokine in mesothelioma showed a response in about 30% of patients when associated with chemotherapy, however with major toxicity [84]. One phase I trial have shown potential therapeutic benefit of IFN-α 2a gene transfer mediated by an adenoviral vector [85] and a new study with this approach is still ongoing (see Table 2).

Intrapleural viruses

Viruses are strong stimulants to the immune system by the activation of the innate as well as the adaptive responses. The measles virus is oncolytic, resulting in tumor cell death and antigen release, allowing T cell priming through dendritic cells. Also, immunoadjuvant properties of the measles virus were shown by loading dendritic cells with measles-infected mesothelioma cells, which resulted in dendritic cell maturation [86]. Phase I clinical trials are under way testing the intrapleural application of measles, herpes and vaccinia virus in patients with malignant pleural mesothelioma (Table 2).

Immune checkpoint inhibition

Cancer cells often inhibit T-cell activation in order to escape immune surveillance. After activation T cells express the cytotoxic T-lymphocyte antigen 4 (CTLA-4). When CTLA-4 binds members of the B7 family the T-cell response becomes abrogated [87.] Blocking monoclonal antibodies have been developed to prevent the negative feedback loop via

CTLA-4. Tremelimumab, is a humanized monoclonal IgG2 antibody binding to CTLA-4. Tremelimumab has been tested as second line in mesothelioma in a phase II trial [88]. Two of 29 patents had durable partial responses. Although the primary endpoint of the study was not met, the disease control rate of 31% and progression-free survival of six months prompted further evaluation in an ongoing randomized phase II study (Table 2). Expression of PD-L1 allows cancers to evade the host immune system by interaction with PD1. Treatment with antibodies targeting these molecules has resulted in extraordinary responses for advanced melanoma and lung cancer. Recently, the expression of PD-L1 it has been demonstrated in mesothelioma [89]. Therefore, therapies targeting this pathway are of major interest and under development for mesothelioma patients.

Mesothelin

Mesothelin is a cell surface glycoprotein expressed in mesothelial and peritoneal cells. Even if the biological function of mesothelin it not fully understood, it is known that mesothelin binds to CA-125 and is involved in cell adhesion (reviewed in [90]). Amatuximab is a chimeric IgG1 antibody targeting mesothelin. Studies demonstrated that it blocks the binding of mesothelin to CA-125 and thus could be used also as a strategy to prevent tumor metastasis [91]. Amatuximab was well tolerated in a phase I trial [92] and currently is tested in phase II in patients with mesothelioma (Table 2). Antibodies can be used to deliver cytotoxic agents to antigen expressing malignant cells. The potent bacterial toxin Pseudomonas exotoxin A (PE38) was linked to a disulfide stabilized variable fragment based on the affinity modified variable light and heavy chain of amatuximab (SS1(dsFv)PE38) and showed pre-clinical activity [93]. SS1(dsFv)PE38 is under clinical investigation and was shown to be safe in two phase I trials, in which 16 patients with mesothelioma were treated. Only minor antitumor activity could be observed. Additionally, the development of neutralizing antibodies was observed in 24% of patients prevented its use for more than one cycle [94]. In a subsequent pilot study using immunosuppressive pre-treatment with pentostatin and cyclophosphamide to

prevent neutralizing antibodies and allow delivery of more courses of treatment, three of ten evaluable patients had major responses [95].

MF-T is a fully humanized anti-mesothelin antibody conjugated to microtubule-targeting toxophore DM4 (BAY 94-9343). It showed selective cytotoxicity against mesothelioma cells, while sparing normal mesothelial cells, and potent in vivo activity against cell line and tumor xenografts [96]. This compound is thus a likely relevant candidate for further clinical testing.

In contrast to these described passive immunological interventions mesothelin can be targeted by active specific vaccination. Live-attenuated Listeria vaccine expressing mesothelin has been tested in a phase I study with mesothelin-expressing tumors. CRS-207 was well tolerated and mesothelin-specific CD8 T cell responses were detected [97]. Recent data presented at ASCO 2014, showed that CRS-207 can be safely combined with standard of care chemotherapy and showed encouraging anti-tumor activity with 9 out of 15 subjects having confirmed durable PR and 4 SD (Table 2).

Wilms tumor suppressor gene 1

The Wilms tumor suppressor gene 1 (WT1), is a transcription factor highly expressed in mesothelioma and WT1 immunohistochemistry is among the routine procedures used for the diagnosis of mesothelioma. WT1 peptides are immunogenic and induce T cell responses against mesothelioma cell lines [98]. In a first clinical trial class I and II WT1 peptides were used for vaccination with subcutaneous GM-CSF, which is used to mature dendritic cells to augment T cell priming. Out of nine patients with mesothelioma treated, one remained without progression after three years and five were documented to have a CD8 T-cell response [99]. Currently, randomized phase II trials with this vaccine are ongoing in patients after completion of multimodality therapy (Table 2).

Vaccination with tumor cell lysate

Mesothelioma cell lysates are used for vaccination and can induce an antitumoral response. Twenty-two patients were treated with autologous tumor cell lysates and GM-CSF. In 32% of the patient an immune response could be induced, but there was no

objective response [100]. One clinical phase I trial is testing an autologous tumor cell vaccine with an adjuvant (ISCOMATRIX) and celecoxib to augment antigen presentation. The tumor cell vaccine is exposed *ex vivo* to demethylating agents to increase expression of tumor antigens (Table 2). Another phase one trial evaluates an allogeneic tumor cell vaccine (K526-GM) in combination with cyclophosphamide and celecoxib (Table 2). Cyclophosphamide is intended to eradicate regulatory T cells, which can inhibit dendritic cells to prime effector T cells [101] . Celecoxib is a COX-2 inhibitor resulting in decreased prostaglandin E2 (PGE2) and was used to block PGE2-mediated conversion of regulatory T cells and to allow dendritic cell maturation [102].

Cellular therapy

Adoptive transfer of dendritic cells pulsed ex vivo with tumor-antigens led to the first FDA approved cellular therapy (Sipuleucel-T) in prostate cancer [103]. In mesothelioma a comparable approach was tested in 10 patients vaccinated with autologous dendritic cells. Each vaccine was composed of mature dendritic cells pulsed with autologous tumor lysate [104]. In four patients, dendritic cell vaccination induced cytotoxic T cells. Results from a trial evaluating dendritic cell-based vaccination in combination with low-dose cyclophosphamide are awaited (Table 2).

T cells can be re-directed against specific antigens. After gene transfer, autologous T cells express a chimeric antigen receptor (CAR), which enables the T cell to destroy target cells. Mesothelin-specific re-directed T cells were developed and showed *in vitro* and *in vivo* activity [105, 106]. To achieve transient expression the plasmid with the gene sequence of the CAR was transferred in the T cells by electroporation [107]. Mesothelin-specific re-directed T cells are tested in early clinical trials (Table 2). An alternative target in malignant mesothelioma is the fibroblast activation protein (FAP) [108, 109]. FAP-specific re-directed T cells demonstrated antigen-specific activity *in vitro* and *in vivo* and are close to early clinical investigation (Table 2) [108, 110] . In this clinical trial the adoptive transfer is planned to be performed into the pleural effusion to overcome blocked T-cells trafficking [111].

Discussion

In contrast to lung cancer, oncogenic driver mutations are absent in malignant mesothelioma. The development of targeted therapy therefore hinges on the exploration of pathways indirectly activated by the loss of tumor suppressor genes or targets associated with the disease phenotype. Efforts of targeting angiogenesis and cancer associated receptor tyrosine kinases have shown disappointing results despite the enrolment of hundreds of mesothelioma patients in clinical trials. Histone deacetylase and proteasome inhibitors were found to be inactive. The promising avenues for targeted therapies in mesothelioma include the functional consequences of alterations in NF2/Hippo pathway, and immunotherapeutic approaches. The inactivation of NF-2 and resulting merlin deficiency leads to a significantly increased activity of several pathways, including the hedgehog pathway, the activity of the focal adhesion kinase (FAK) and the PI3K/mTOR pathway. Inhibition of these pathways resulted in reproducible growth reduction of mesothelioma in preclinical models. Phase I trials in mesothelioma demonstrated clinical activity of FAK and of PI3K/mTOR inhibitors and a randomized phase II trial of the FAK inhibitor defactinib as maintenance therapy after chemotherapy has been initiated. In regard to immunotherapeutic approaches the jury is still out. However, based on early results in non-small cell lung cancer and other solid tumors it appears likely that immune checkpoint inhibitors will find a place in the therapy of mesothelioma.

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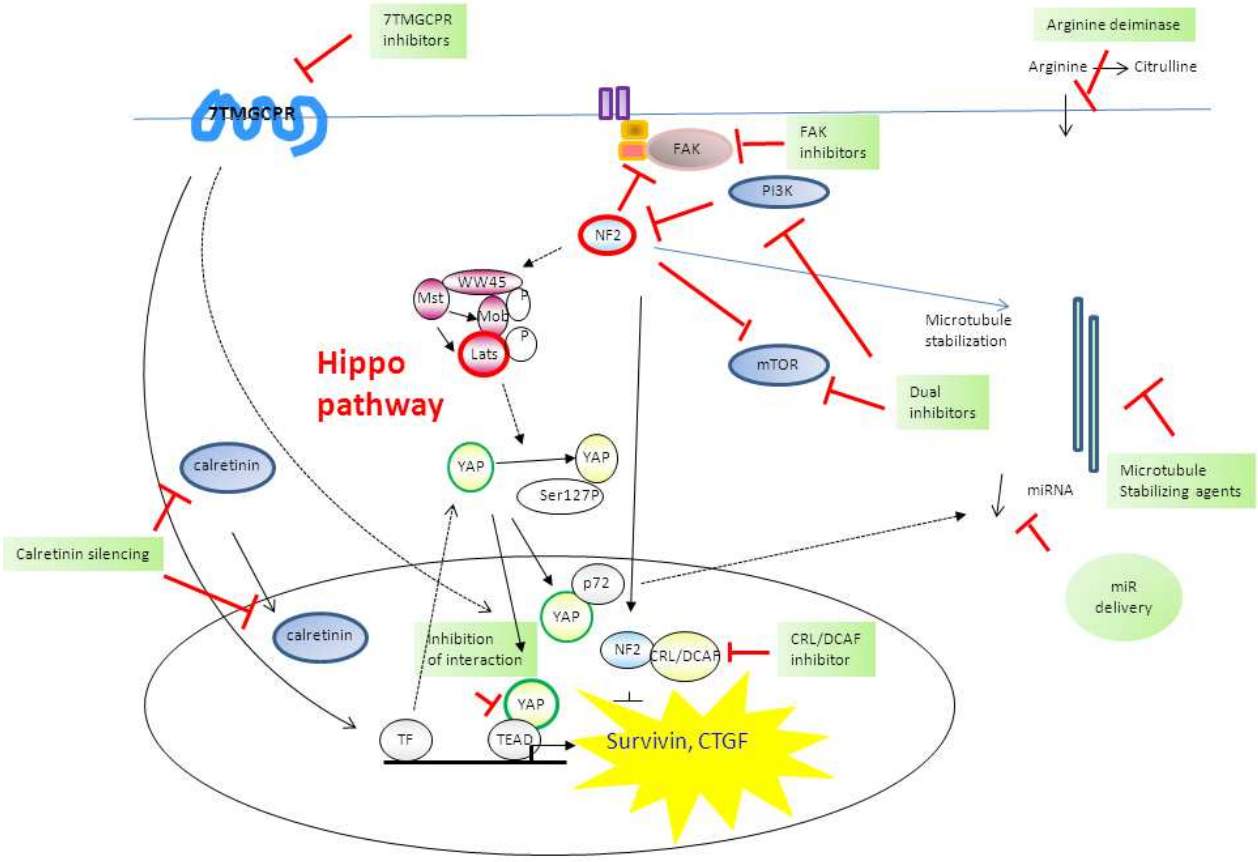
Figure legends

Figure 1. Genetic/epigenetic changes present in mesothelioma offer different possibilities for therapeutic intervention. Functional inactivation of NF2 and NF2/Hippo pathway offers the opportunity to intervene using focal adhesion kinase (FAK) inhibitors, PI3/mTOR dual inhibitors, microtubule stabilizing agents, E3 ubiquitin ligase CRL4/DCAF inhibitors, tumor suppressor miR delivery, inhibitors of the interaction between YAP and TEAD transcription activators, and 7 transmembrane G-coupled receptors (7TMGCPR) inhibitors regulating YAP activity. Tumor promoting activity of high mobility group-box-1 (HMGB1) can be blocked using HMGB1 antibodies. Auxotrophy for arginine render the tumor sensitive to the activity of arginine deiminase. Calretinin-dependent survival can be blocked by calretinin silencing.

Figure 2. Interplay of the immune system with malignant mesothelioma and possible immunotherapeutic interventions. Malignant mesothelioma cells express tumor antigens like WT1, FAP, mesothelin. Tumor-antigens or other components from the malignant cells can be picked up by dendritic cells and presented to T cells inducing a T-cell response. However, molecules like TGF-β can block T cell priming in the lymph node or T cell function in malignant tissue. T cell function can be also abrogated by activation of CTLA-4 and PD-1 on primed T cells. In addition, effector T cells can be converted in immunosuppressive regulatory T cells. Dendritic cell and re-directed T cells can be adoptively transferred. Dendritic cells loaded with peptides derived from tumor-antigen induce T cells response. To enhance priming GM-CSF is given in combination with dendritic cells. To circumvent T-cells priming re-directed effector T cells can be transferred. IFN-γ is used to augment MHC expression on tumor cells increasing immunogenicity.



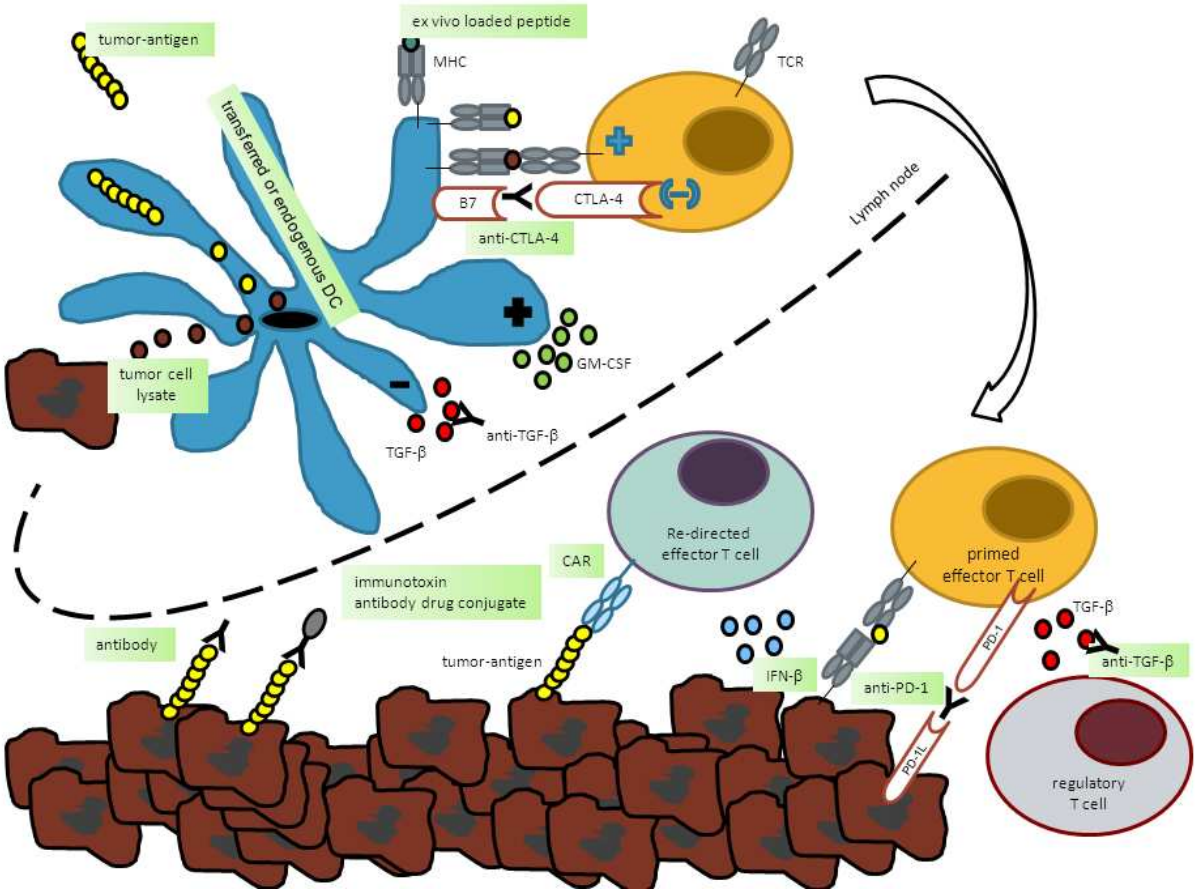


Table 1 Clinical trials investigating targeted therapy in mesothelioma

target	clinical trial ID	experimental arm	mechanism	control arm	phase	primary endpoints	expected completion date
chemotherapy sensitizer	NCT00700336	CBP501 plus Cis/Pem	G2 checkpoint inhibition	Cis/Pem	I/II	safety, MTD, PFS	completed
altered NF2 signaling	NCT01138033	GSK2256098	Focal Adhesion Kinase inhibitor		I	MTD	December 2014
	NCT01870609	VS-6063	Focal Adhesion Kinase inhibitor	Placebo	II	OS, PFS	December 2016
	NCT02004028	VS-6064	Focal Adhesion Kinase inhibitor		II	PK	November 2014
altered NF2 signaling	NCT00854152	GDC-0980	PI3K/mTOR inhibitor		I	PK, MTD	August 2014
	NCT01655225	LY3023414	PI3K/mTOR inhibitor		I	Recommended Phase 2 dose	September 2014
altered NF2 signaling	NCT00770120	Everolimus	mTOR inhibitor		II	PFS, RR	completed
	NCT01024946	Everolimus	mTOR inhibitor		II	PFS, RR	completed
arginine dependency	NCT01279967	Pegylated Arginine Deiminase	Growth inhibition of ASS negative tumors	Best supportive care	II	PFS	January 2014
receptor tyrosine kinases	NCT00402766	Imatinib mesylate plus Cis/Pem	bcr/abl, c-kit and PDGFR TKI		I	MTD	August 2015
	NCT00703638	Sorafenib plus Cis/Pem	VEGFR2, VEGFR3, Raf, PDGFR, and c-kit TKI		I	MTD	Completed
	NCT01064648	Cediranib plus Cis/Pem	VEGFR 1-3	Placebo (phase II)	I/II	MTD	June 2014
	NCT00700336	Dasatinib	Src inhibition and PDGFR TKI		I	modulation of p-Src Tyr419	March 2016
	NCT01592383	Erlotinib	EGFR TKI		II	RR	June 2015
	NCT00459862	Pazopanib	VEGFR1-3, PDGFR and c-Kit		II	PFS	completed
	NCT01211275	Axitinib	pan-VEGFR inhibitor	Cis/pem	I/II	safety/efficacy	unknown
	NCT01861301	Tivantinib	MET inhibitor		II	ORR	February 2015
	NCT02049060	Tivantinib plus Carbo/Pem	MET inhibitor		I	DLTs	July 2014
	NCT01590160	Ganetespib plus Cis/Pem	Hsp90 inhibitor		I/II	DLTs	December 2015
	NCT01307100	Nintedanib plus Cis/Pem	VEGFR, PDGFR and FGFR TKI	Cis/Pem	II	PFS	May 2016
	NCT01769547	Dovitinib	FGFR3 inhibitor		II	PFS	June 2016
proteasome	NCT00996385	Bortezomib and oxaliplatin	proteasome inhibitor		II	RR	September 2013
aminopeptidase N	NCT01358084	NGR-hTNF	TNF targeting tumor blood vessels	Best supportive care	II	PFS	June 2013

Table 2 Clinical trials investigating immunotherapy in mesothelioma

Target	clinical trial ID	experimental arm	mechanism	control arm	phase	primary endpoints	expected completion date
immunosuppressive cytokine	NCT01112293	anti-TGF-β monoclonal antibody (GC1008)	Blocking TGF-β		II	PFS	October 2012
immunomodulating cytokine	NCT01212367	Gene transfer IFN-alfa 2a	immunomodulating cytokine		I	safety	December 2027
immunoadjuvant	NCT01503177	intrapleural measles virus	Dendritic cell maturation; oncolytic virus		I	safety	September 2014
	NCT01721018	intrapleural herpes virus	Oncolytic virus		I/II	safety, PFS	April 2014
	NCT01766739	intrapleural vaccinia virus (GL-ONC1)	Oncolytic virus		I	safety	January 2015
immune checkpoint	NCT01843374	Tremelimumab	Blocking CTLA-4	Placebo	II	OS	May 2016
tumor-antigen passive	NCT00738582	MORAb-009 (Amatuximab)	anti-Mesothelin monoclonal antibody with With pemetrexed and cisplatin		II	PFS	November 2014
tumor-antigen active-specific	NCT01675765	CRS-207 live-attenuated Listeria vaccine expressing mesothelin	active-specific immune response against mesothelin followed pemetrexed and cisplatin		I	safety	December 2015
	NCT01265433	WT-1 analog peptide Vaccine plus GM-CSF	adjuvant, active-specific immune response against WT-1	Montanide adjuvant + GM-CSF	II	PFS	December 2014
	NCT01890980	WT-1 analog peptide Vaccine plus GM-CSF	adjuvant, active-specific immune response against WT-1	Montanide adjuvant + GM-CSF	II	PFS	December 2017

	NCT01258868	Autologous tumor cell vaccine	active-specific immune response against autologous tumor cells in combination with celecoxib and ISCOMATRIX		I	safety	November 2018
	NCT01143545	Allogeneic tumor cell vaccine	active-specific immune response against allogeneic tumor cells in combination with cyclophosphamide and celecoxib		I	safety	May 2017
	NCT01569919	TroVax: pox virus specific for antigen 5T4	Pox virus carrying the 5T4 antigen plus Cis/Pem		II	Immune responses to 5T4 safety	June 2014
	NCT00280982	tumor lysate-loaded autologous dendritic cells	active-specific immune response against autologous tumor cells		I	safety	completed
tumor-antigen active-specific adoptive transfer	NCT01241682	tumor lysate-loaded autologous dendritic cells low-dose cyclophosphamide	active-specific immune response against autologous tumor cells		I	safety	completed
tumor-antigen adoptive transfer	NCT01583686	Adoptive transfer of mesothelin-specific re-directed T cells	T cell response		I/II	safety/PFS	March 2019
	NCT01355965	Adoptive transfer of mesothelin-specific re-directed T cells	T cell response		I	safety	May 2014
	NCT01722149	Adoptive transfer of FAP-specific re-directed T cells	T cell response		I	safety	May 2015